

PCT

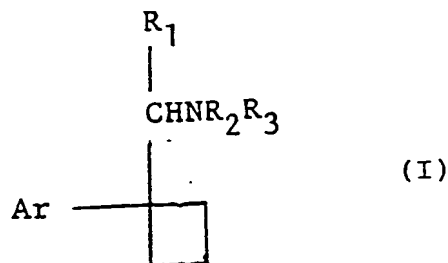
WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification<sup>4</sup> : A61K 31/135, 31/395, 31/215 A61K 31/33, 31/195</p>	<p>A1</p>	<p>(11) International Publication Number: <b>WO 88/ 06444</b> (43) International Publication Date: 7 September 1988 (07.09.88)</p>
<p>(21) International Application Number: PCT/GB88/00129 (22) International Filing Date: 25 February 1988 (25.02.88) (31) Priority Application Number: 8704777 (32) Priority Date: 28 February 1987 (28.02.87) (33) Priority Country: GB (71) Applicant: THE BOOTS COMPANY PLC [GB/GB]; 1 Thane Road West, Nottingham NG2 3AA (GB). (72) Inventor: REES, John, Andrew ; 50 First Road, Edwalton, Nottinghamshire (GB). (74) Agent: THACKER, Michael, Anthony; The Boots Company PLC, Patent Department, R4 Pennyfoot Street, Nottingham NG2 3AA (GB).</p>		<p>(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).  Published With international search report.</p>

(54) Title: ARYLCYCLOBUTYL DERIVATIVES FOR TREATMENT OF PARKINSON'S DISEASE



(57) Abstract

Compounds of formula (I) in which Ar is optionally substituted phenyl, R<sub>1</sub> is an optionally substituted aliphatic group and R<sub>2</sub> and R<sub>3</sub> are H or optionally substituted alkyl groups or R<sub>2</sub> and R<sub>3</sub> together with the nitrogen atom to which they are attached complete a heterocyclic ring, are used in the treatment of Parkinson's disease. The compounds of formula (I) may be administered with a dopamine precursor such as levodopa and/or a dopa decarboxylase inhibitor such as carbidopa or benserazide. A preferred compound of formula (I) is *N,N*-dimethyl-1-[1-(4-chlorophenyl)-cyclobutyl]-3-methylbutylamine hydrochloride monohydrate.

***FOR THE PURPOSES OF INFORMATION ONLY***

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

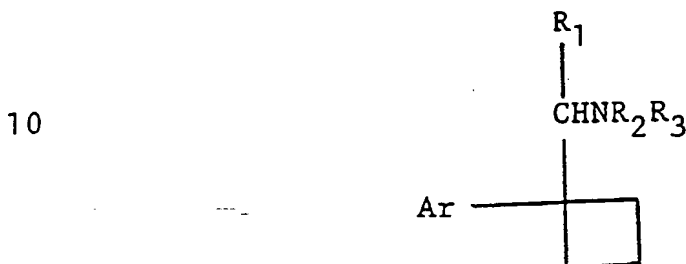
AT	Austria	FR	France	ML	Mali
AU	Australia	GA	Gabon	MR	Mauritania
BB	Barbados	GB	United Kingdom	MW	Malawi
BE	Belgium	HU	Hungary	NL	Netherlands
BG	Bulgaria	IT	Italy	NO	Norway
BJ	Benin	JP	Japan	RO	Romania
BR	Brazil	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	LI	Liechtenstein	SN	Senegal
CH	Switzerland	LK	Sri Lanka	SU	Soviet Union
CM	Cameroon	LU	Luxembourg	TD	Chad
DE	Germany, Federal Republic of	MC	Monaco	TG	Togo
DK	Denmark	MG	Madagascar	US	United States of America
FI	Finland				

- 1 -

## Arylcyclobutyl derivatives for treatment of Parkinson's disease.

This invention relates to the medical treatment of Parkinson's disease which is due to degenerative changes in the ganglia at the base of the cerebrum.

- 5 According to the present invention there is provided a method of treating Parkinson's disease in which a therapeutically effective amount of a compound of formula I



- 15 in which Ar is optionally substituted phenyl,  $R_1$  is an optionally substituted aliphatic group or a carbocyclic or heterocyclic group and  $R_2$  and  $R_3$  are H or optionally substituted alkyl groups or  $R_2$  and  $R_3$  together with the nitrogen atom to which they are attached complete a heterocyclic ring

- 20 is administered in conjunction with a pharmaceutically acceptable diluent or carrier. The compound of formula I may be administered with a dopamine precursor such as levodopa and/or a dopa decarboxylase inhibitor such as carbidopa or benserazide

- 25 Suitable compounds of formula I are described in British Patents 2098602, 2127819 and 2128991 and in European Patent Application 191542 and may be used in the forms of pharmaceutically acceptable salts and in the form of solvates. A particularly preferred

- 2 -

compound of formula I is N,N-dimethyl-1-[1-(4-chloro-phenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate which is described in European Patent Application 230742.

5           Compounds of formula I which cause an increase in dopamine function have utility in the treatment of Parkinson's disease. N,N-Dimethyl-1-[1-(4-chloro-phenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate is an inhibitor of dopamine reuptake and  
10 when administered to humans gives an increase in dopamine levels in plasma. It may be used alone in the treatment of Parkinson's disease or may be used in combination with a dopamine precursor such as levodopa and/or a dopa decarboxylase inhibitor such as carbidopa  
15 or benserazide.

          Compounds of formula I may be administered in any of the known pharmaceutical dosage forms for example solid dosage forms such as tablets or capsules or liquid dosage forms for example those forms intended  
20 for oral or parenteral administration. The amount of the compound of formula I to be administered will depend on a number of factors including the age of the patient, the severity of the condition and the past medical history of the patient and always lies within  
25 the sound discretion of the administering physician but it is generally envisaged that the dosage of the compound of formula I to be administered will be in the range 1 to 1000 mg preferably 5 to 500 mg per day given in one or more doses. When the compound of formula I  
30 is administered with levodopa the amount of levodopa given will be progressively increased by the physician until an optimum response is obtained. The actual amount will be under the control of the physician and may be up to 8 g per day given in divided doses. When  
35 the compound of formula I is administered with

carbidopa the amount of carbidopa given will be up to 100 mg per day. When the compound of formula I is administered with benserazide the amount of benserazide given will be up to 200 mg per day.

5       The ability of the compound to inhibit reuptake of dopamine is demonstrated by the following techniques.

1)   In vitro inhibition of dopamine uptake

Male Sprague-Dawley rats (Charles River) were killed by cervical dislocation and the brains removed and placed in an ice-cold oxygenated Krebs solution containing 120mM NaCl, 4.7mM KCl, 2.1mM  $\text{KH}_2\text{PO}_4$ , 1.2mM  $\text{CaCl}_2$ , 0.6mM  $\text{MgSO}_4$ , 25mM  $\text{NaHCO}_3$  and 11mM glucose. The brains were then dissected according to the method of Glowinski and Iversen [J. Neurochem. (1966) 13 655-669] and the striata removed. The samples of striata were pooled, weighed and transferred to a glass homogenising vessel on ice, containing oxygenated 0.32M sucrose solution (20 volumes). The striata were homogenised with six strokes of a ptfе pestle having a clearance of 0.35 mm (manufactured by TRI-R Homogenisers Ltd.). The homogenate was centrifuged at 1000 x g for 10 minutes at 4°C and the supernatant containing a suspension of synaptosomes was used in the dopamine uptake inhibition tests described below. Polythene specimen tubes containing 1.5 ml Krebs solution, 0.2 ml of a solution of N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate or distilled water as control, and 0.1 ml of the synaptosome suspension were provided with an atmosphere of 5% carbon dioxide and 95% oxygen and pre-incubated at 37°C for 5 minutes. A solution of  $^{14}\text{C}$ -dopamine hydrochloride (Amersham International) was added (0.2 ml) to give a final concentration of 0.17µM. The incubation was continued for a further 5 minutes before

- 4 -

the contents of the tubes were filtered under vacuum through Whatman GF/F filters which were washed with ice-cold Krebs solution (2 x 5 ml). The filters were placed in scintillation vials containing 10 ml scintillation fluid (ES-299 supplied by Packard Instruments) and the radioactivity in the vials counted on a Packard 4530 scintillation counter. In each experiment there was a control tube in which no test compound was present, 3 tubes in which the compound being tested was present at one of three concentrations (100, 10 and 1 $\mu$ M) and a background tube containing no test compound which was maintained at 0°C to determine passive <sup>14</sup>C-dopamine uptake. The count for each tube was registered in counts per minute (cpm) and the % inhibition of uptake (I) calculated from the formula

$$I = 100 \times \frac{\text{mean cpm for control} - \text{mean cpm for test}}{\text{mean cpm for control} - \text{mean cpm for background}}$$

The results obtained in three replicate experiments are set out below. The test compound inhibited <sup>14</sup>C-dopamine uptake in a concentration-dependent manner. The concentration which gave 50% inhibition of uptake was then calculated and is given below as the IC50 figure for each experiment. The mean ( $\pm$ SEM) IC50 value for inhibition of dopamine uptake by the test compound is  $11 \pm 4.2 \mu\text{M}$ .

- 5 -

	Concentration in Test Tube ( $\mu$ M)	% Inhibition of uptake		
		Ex.1	Ex.2	Ex.3
	100	96	81	86
	10	70	35	48
5	1	31	4	12
	IC50	3.3 $\mu$ M	18 $\mu$ M	11 $\mu$ M

The results show that N,N-dimethyl-1-[1-(4-chloro-phenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate inhibited the uptake of dopamine into striatal synaptosomes in vitro.

## 2) In vivo inhibition of dopamine reuptake

An in vivo test for dopamine reuptake inhibition relies on the fact that such reuptake inhibitors can prevent the entry of dopamine-depleting agents into neurons. Depleting agents interfere with the neuronal storage mechanism for dopamine so dopamine leaks into the cytoplasm where it is metabolised by monoamine oxidase. Depleting agents therefore induce a large reduction in brain dopamine levels which can be measured experimentally. Prior treatment with a dopamine reuptake inhibitor reduces the depletion of dopamine levels caused by subsequent administration of a depleting agent such as  $\alpha$ -methyl-m-tyrosine.

Male Sprague-Dawley rats (180-220 g; Charles River) were randomly assigned to various treatment groups. Two groups were dosed orally with vehicle (distilled water) and the remaining groups were given oral doses of N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate at

- 6 -

3 different doses. Thirty minutes later, one vehicle treated group and all the test groups were given an i.p. injection (2 ml/kg) of the depleting agent  $\alpha$ -methyl-m-tyrosine (25 mg/kg; Sigma Chemical Co.).

- 5 The group receiving vehicle (p.o.) plus  $\alpha$ -methyl-m-tyrosine (i.p.) served as the depleted control. The remaining vehicle treated group was injected with saline i.p. to act as the absolute control.

- 10 Four hours after the i.p. injections the animals were sacrificed and the whole brains rapidly removed and frozen on dry-ice. The samples were stored at  $-30^{\circ}\text{C}$  prior to determination of dopamine concentrations.

- 15 Brain samples were thawed and homogenised in 4 volumes of 0.4M perchloric acid containing sodium metabisulphite (0.4mM) and the internal standard 3,4-dihydroxybenzylamine (0.8 $\mu\text{M}$ ). The samples were homogenised using a Polytron homogeniser on speed setting 6 for 10 seconds, after which they were  
20 centrifuged at 23000 x g for 10 minutes at  $4^{\circ}\text{C}$  using a Sorvall RC-5B centrifuge and SM-24 rotor. Supernatants were removed and their dopamine concentration determined using an HPLC (high pressure liquid chromatography) system with fluorimetric detection.

- 25 The percentage prevention (P) of depletion of brain dopamine levels by test compounds is calculated from the formula

$$30 \quad P = 100 \times \frac{\text{Control} - \text{Test compound/depletor}}{\text{Control} - \text{deionised water/depletor}}$$



- 7 -

The test compound exhibited a dose-dependent prevention of brain dopamine depletion. From the percentage prevention values obtained at three doses an oral ED<sub>50</sub> dose was calculated, that is, the dose to prevent  
5 depletion of brain dopamine by 50%. The ED<sub>50</sub> for N,N-dimethyl-1-[1-(4-chlorophenyl)cyclo-butyl]-3-methylbutylamine hydrochloride monohydrate was calculated as 44 mg/kg (p.o.).

10 3) Inhibition of dopamine uptake in vitro by plasma from drug treated rats

It has been demonstrated that the plasma of rats which have been treated with N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine  
hydrochloride monohydrate inhibits the uptake of  
15 radiolabelled dopamine into freshly prepared synaptosomes from rat striatum.

To obtain the synaptosomes untreated male CD rats (Sprague-Dawley 200-250g; Charles River) were killed by cervical dislocation, the brains removed and the  
20 striata of two or three rats dissected out and placed in ice-cold saline. The pooled tissue was homogenised in 20 volumes of ice-cold 0.32M sucrose in a glass-teflon homogeniser with 0.35 mm clearance. The homogenate was spun at 1500 x g for 10 minutes in a  
25 refrigerated (4°C) Heraeus Christ minicentrifuge. The supernatant was transferred into a polypropylene sterilin tube and stored on ice for as short a time as possible prior to use in the radiolabelled dopamine uptake assay.

30 Male CD rats (Sprague-Dawley 350-400g; Charles River) were given 10, 30 or 100 mg/kg of N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate which was dissolved in

- 8 -

distilled water and administered orally at a rate of 5 ml/kg. Control rats were orally administered distilled water. After one hour the rats were killed by CO<sub>2</sub> inhalation overdose, the chest cavity opened and  
5 blood immediately removed from the heart and mixed with an anticoagulant solution.

The rat blood/anticoagulant mixture was spun at 5750 x g for 20 minutes in a Heraeus Christ minicentrifuge at room temperature. The plasma was  
10 removed and kept at room temperature for as short a time as possible before analysis in the radiolabelled dopamine uptake assay. The fresh plasma samples from the rat study were initially maintained at 37°C for 10-15 minutes before starting the assay. 300 µl of  
15 each plasma sample was added to six 5 ml polypropylene tubes already containing 50 µl of saline at room temperature. Four of these tubes were incubated at 37°C to assess active radiolabelled dopamine uptake. The other two tubes were immediately cooled to c.3°C  
20 and maintained at this temperature to account for passive radiolabelled dopamine uptake.

a) Active uptake of radiolabelled dopamine

To each tube at 37°C was added 100 µl of fresh striatal synaptosomes, prepared as above. These tubes  
25 were then agitated in the water bath at 37°C for 5 minutes prior to the addition of 50 µl ice-cold <sup>3</sup>H-dopamine solution (final concentration 1 x 10<sup>-7</sup>M) (Amersham International). The reaction was stopped after a further 5 minutes of agitation and incubation  
30 at 37°C by removing the tubes from the water bath and immediately adding 4 ml of ice-cold saline. The contents of the tube were then rapidly filtered over Whatman glass fibre (GF/F) filters supported on a Millipore 1225 manifold linked to an Edwards 2-stage

vacuum pump. The tubes were rinsed with 2 x 4 ml of ice-cold saline and this was also rapidly filtered. Finally, each manifold well was washed with 4 ml of ice-cold saline.

5    b)    Passive uptake of radiolabelled dopamine

To the two tubes maintained at c.3°C was added 100 µl of fresh striatal synaptosomes followed by 50 µl of ice-cold  $^3\text{H}$ -dopamine solution (final concentration  $1 \times 10^{-7}\text{M}$ ). The "reaction" in these tubes was  
10 terminated by the addition of 4 ml ice-cold saline and the samples were then rapidly filtered and washed as described in (a) above.

All filters were placed into glass vials and 10 ml of Packard ES-299 scintillation fluid added. Filters  
15 were allowed to solubilise for at least 1 hour before the radioactivity accumulated was assayed by liquid scintillation counting.

c)    Calculation of percentage inhibition of  
          $^3\text{H}$ -dopamine uptake

20        The amount of passive uptake of  $^3\text{H}$ -dopamine at c.3°C (measured in cpm) was subtracted from the amount of active  $^3\text{H}$ -dopamine uptake at 37°C (measured in cpm) to derive net  $^3\text{H}$ -dopamine uptake. The resulting value was then expressed as a percentage (X) of the net  
25  $^3\text{H}$ -dopamine uptake recorded for control plasma. The latter plasma samples were obtained from distilled water-treated control rats. The percentage inhibition value (X) was calculated using the following formula:

- 10 -

$$X=100 \times \frac{(\text{CONTROL a} - \text{CONTROL p}) - (\text{TREATED a} - \text{TREATED p})}{(\text{CONTROL a} - \text{CONTROL p})}$$

a = active

p = passive

- 5 Mean [ $\pm$  1 standard error of the mean (SEM)] percentage inhibition was then determined for each dose.

The results obtained for 12 different rats (4 rats at each dose) are given below.

Dose of % Inhibition of dopamine uptake							
10	Drug	in Individual Rats				Mean ( $\pm$ SEM)	
	100	53	59	67	55	58.5	$\pm$ 3.1
	30	54	35	49	42	45.0	$\pm$ 4.1
	10	33	23	13	17	22.8	$\pm$ 5.5

- 15 These results clearly show that plasma from rats administered N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate demonstrates a dose-dependent inhibition of radiolabelled dopamine uptake into rat striatal synaptosomes.

- 20 4) Inhibition of dopamine uptake in vitro by plasma from drug treated rats obtained over a period of time after a single 30 mg/kg dose

- 25 The percentage inhibition of dopamine uptake was determined in a similar manner to that described in (3) above. Plasma was obtained from different animals at various periods after oral administration of a dose of

- 11 -

30 mg/kg of N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate. The results obtained are set out below:

5	Time after dosing (hrs)	% Inhibition of dopamine uptake	
		- Mean ( $\pm$ SEM)	
	1	45.0 $\pm$ 4.1	
	3	63.0 $\pm$ 1.7	
	8	73.4 $\pm$ 3.5	
10	24	31.5 $\pm$ 2.4	
	48	19.5 $\pm$ 5.2	
	72	10.4 $\pm$ 1.6	

These results show that the ability of rat plasma to inhibit the uptake of dopamine into synaptosomes from rat striata persists for a considerable period of time after dosing with drug.

5) Inhibition of dopamine uptake in vitro by plasma from humans treated with drug

It has been demonstrated that the plasma obtained from human volunteers administered N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate inhibits the uptake of radiolabelled dopamine into freshly prepared synaptosomes from rat striatum which were obtained as described in (3) above.

Venous blood (100 ml) was collected immediately before an oral 50 mg dose of N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate was administered to six

- 12 -

healthy human volunteers and a further venous blood sample was taken 3 hours later. The plasma was separated by centrifugation at 2900 x g for 20 minutes at room temperature in a Heraeus Christ minicentrifuge and was stored at -20°C prior to analysis. The samples were thawed in a water bath at 37°C for 10-15 minutes prior to commencement of the in vitro dopamine uptake assay which was performed, as described in (3) above except <sup>14</sup>C-dopamine was used. The assay was performed twice on plasma from each volunteer. The results obtained are given below:

	Volunteer	% Inhibition of dopamine uptake		Mean
15	1	21.6	1.5	11.6
	2	15.5	1.6	8.6
	3	13.4	17.9	15.7
	4	9.2	26.6	17.9
	5	13.0	18.4	15.7
	6	9.9	22.3	16.1

20 - The mean ( $\pm$ SEM) % inhibition of <sup>14</sup>C-dopamine uptake by plasma from the six drug-treated volunteers was 14.2  $\pm$  1.4.

25 6) Dopamine reuptake inhibition demonstrated by ipsilateral circling behaviour of unilateral nigrostriatal lesioned rats following drug administration.

The two tracts of the nigrostriatal dopamine system are independent and are located on either side

- 13 -

of the midline of the brain. When one tract is destroyed using the specific neurotoxin, 6-hydroxy-dopamine (6-OHDA), rats will display characteristic circling behaviour after injection of dopaminergic drugs. The direction of rotation, however, is dependent on the stimulus employed. Drugs which inhibit dopamine reuptake can only function on the unlesioned side of the brain and induce circling towards the lesion site (known as ipsilateral circling.)

Male CD rats (250-300g; Charles River) were anaesthetised with 'Equithesin' type anaesthetic (3.2 ml/kg i.p.) and secured in a stereotaxic frame (David Kopf Small Animal Stereotaxic Instrument DKI 900). After shaving the area a sagittal incision of 1.5-2 cm was made and skin flaps dissected from the skull. A small hole was made in the skull, using a No. 6 dental burr, to allow the tip of a 30 s.w.g. stainless steel cannula to be inserted to the left substantia nigra. This was located by using the following co-ordinates, using skull landmark bregma as the zero reference point; rostral-caudal -2.8; medial-lateral +2.0; dorsal-ventral -8.0 from the surface of the dura, all co-ordinates measured in millimetres. This system of co-ordinates is a modification of the de Groot system as described by Pellegrino et al (A stereotaxic atlas of the rat brain, 2nd Edition, Plenum Press 1979). 6-Hydroxydopamine HBr (2 µg/µl, as base; Sigma Chemical Co.) was injected into the left substantia nigra at 1 µl/min; a total of 8 µg was administered over a period of 4 minutes using an infusion pump (Braun 'Perfusor' ED2). After removal of the cannula the skin flaps were joined with a single everted suture and the animal allowed to recover.

- 14 -

After 21 days, the circling behaviour of the rats was examined. They were placed, individually, in circular plastic arenas (30 cm diameter x 12 cm high) for 1 hour, with no access to food or water during this period. Every 10 minutes each animal was observed for 1 minute and the number of 'turns' counted. One 'turn' consisted of rotation through 360° in either direction. As rats were always lesioned on the left side of the brain, anticlockwise turns were ipsilateral.

Control values were determined for all rats by observing their spontaneous circling behaviour in the arenas without prior dosing. The mean turns per minute was always less than 1.

The rats were challenged with an intraperitoneal dose of methamphetamine (2 mg/kg) and immediately placed in the arenas. In these experiments circling behaviour was monitored during two periods, 0-1h and 4-5h after dosing. Rats giving a mean of more than 5 ipsilateral turns per minute during the first hour in response to methamphetamine were used in subsequent tests. Following selection, the rats were used in groups of 5 or more rats to test the drug under investigation. The groups were made up of rats exhibiting varying responses to methamphetamine (always >5 turns/min as stated above), the mean response of the group was always more than 10 ipsilateral turns per minute.

N,N-Dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate was administered at 30 mg/kg orally and the amount of circling observed at various time periods after dosing is set out below:



- 15 -

	time (hrs)	ipsilateral turns/min
		(Mean $\pm$ SEM)
5	0-1	3.0 $\pm$ 0.6
	4-5	6.7 $\pm$ 1.4
	8-9	8.6 $\pm$ 1.8
	24-25	6.2 $\pm$ 1.7
	48-49	1.7 $\pm$ 0.5

These results indicate that at this dose N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate has a long lasting action as an inhibitor of dopamine reuptake.

7) Measurement of the turnover of dopamine in rodent brains by determination of DOPAC concentrations following drug administration

Inhibition of dopamine reuptake in the brain reduces the rate at which dopamine is synthesised and metabolised (the turnover rate). This can be assessed by measuring the amount of the dopamine metabolite DOPAC (dihydroxyphenylacetic acid) which accumulates in the brains of rats and mice. In addition, the administration of probenecid blocks active transport of DOPAC out of the brain. The subsequent rise in brain DOPAC concentrations is attenuated by drugs which inhibit dopamine reuptake.

N,N-Dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate (10 mg/kg) was administered orally to male Sprague-Dawley rats (180-250g) or male CD1 mice (25-30g) (Charles River). One group of animals was killed two hours later. An

- 16 -

additional group was administered probenecid (200 mg/kg i.p.; Sigma Chemical Co.) 30 minutes after the drug and then killed after a further 90 minutes. The animals were killed by decapitation and brains were rapidly removed and dissected on an ice-chilled porcelain plate. They were cut longitudinally along the midline; one half was retained for whole brain analysis while the striatum was dissected from the other. Tissues were immediately frozen in liquid N<sub>2</sub> and stored over dry ice (-80°C) until assay. Whole brain tissue was homogenised in 5 volumes 0.4M perchloric acid (containing 0.01% (w/v) Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, 0.1% (w/v) EDTA) and striata in 600 µl using a Polytron (setting 6; 20 seconds) fitted with a microprobe (PT-7). Samples were then centrifuged at 30,000 x g (whole brain samples) or at 3,500 x g (striata) using a microfuge (Beckman) set up to take 1200 µl polypropylene tubes. Aliquots (50 µl) of the clear supernatants were then injected automatically into the chromatographic system for the separation and quantification of DOPAC.

High pressure liquid chromatography (HPLC) combined with electrochemical detection (ECD) was employed to assay DOPAC. A mobile phase (0.1M Na<sub>2</sub>HPO<sub>4</sub>: CH<sub>3</sub>OH (84:16%) containing 0.1% octanesulphonic acid, 0.1% EDTA and 0.01% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) was delivered by a Dupont 870 pump module at a flow-rate of 1.0 ml/min to a reverse-phase analytical column (25 x 0.4 cm) and guard column (both packed with 5 µm Spherisorb ODS 2) maintained at 45°C in a thermostatically controlled cabinet. Automatic sample injection was provided by a WISP 710B module (Waters Associates) and ECD was performed using a Bioanalytical Systems LC4A controller and cell, with a glassy carbon electrode maintained at +0.65v versus a Ag-AgCl reference electrode. The controller was set at 20nA full-scale and output from

- 17 -

the cell recorded using a Spectra-Physics 4100 automatic computing integrator. Quantification of DOPAC was effected by the computing integrator after calibration of the HPLC-ECD system using DOPAC of known concentration and including isoprenaline as an internal standard.

The results obtained for the effect of the drug on brain DOPAC levels are set out below. Experiments marked A show the ability of drug alone to reduce brain DOPAC concentrations. Experiments marked B show the ability of the drug to attenuate the probenecid-induced elevation of brain DOPAC concentrations.

				DOPAC Levels (ng/g wet wt)	
				Control	Drug
15	A	mouse	whole	74 ± 5	54 ± 3
	A	rat	whole	62 ± 5	36 ± 4
	A	rat	striatum	921 ± 56	601 ± 17
	B	mouse	whole	116 ± 8	54 ± 3
	B	rat	whole	169 ± 3	130 ± 9
	B	rat	striatum	1325 ± 79	920 ± 70

The decreases in brain DOPAC concentrations are indicative of dopamine reuptake inhibition causing a decrease in dopamine turnover in drug treated animals.

The ability of N,N-dimethyl-1-[1-(4-chloro-phenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate to increase the dopamine level in the plasma of human subjects to which the compound had been administered was illustrated by the following trial.

- 18 -

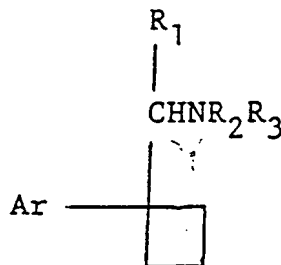
The amount of dopamine in the plasma of six humans subjects who had received a single dose of 30 mg N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate per day for seven days was measured in plasma samples taken two hours after administration. The mean value of dopamine in picograms/ml is set out below at days 1, 4 and 7. Eight human subjects who were given placebo tablets provides plasma samples from which the mean control dopamine levels given below were obtained.

	<u>Dopamine levels (pg/ml)</u>		
	<u>Day 1</u>	<u>Day 4</u>	<u>Day 7</u>
Control	122	110	118
Treated	254	294	219

These figures clearly show that the plasma dopamine levels had increased in the human subjects to which N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate at a dose of 30 mg had been administered.

## CLAIMS:

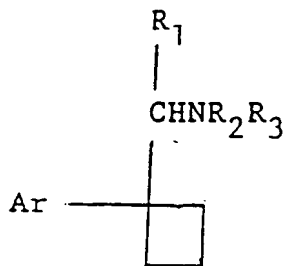
1. The use of a compound of formula I



in which Ar is optionally substituted phenyl,  $R_1$  is an optionally substituted aliphatic group and  $R_2$  and  $R_3$  are H or optionally substituted alkyl groups or  $R_2$  and  $R_3$  together with the nitrogen atom to which they are attached complete a heterocyclic ring for the manufacture of a medicament for the treatment of Parkinson's disease.

2. The use according to claim 1 wherein the compound of formula I is N,N-dimethyl-1-[1-(4-chlorophenyl)-cyclobutyl]-3-methylbutylamine hydrochloride monohydrate.

3. A pharmaceutical composition for the treatment of Parkinson's disease comprising a pharmaceutically effective amount of a compound of formula I



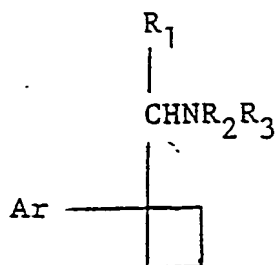
- 20 -

in which Ar is optionally substituted phenyl,  $R_1$  is an optionally substituted aliphatic group and  $R_2$  and  $R_3$  are H or optionally substituted alkyl groups or  $R_2$  and  $R_3$  together with the nitrogen atom to which they are  
 5 attached complete a heterocyclic ring and a pharmaceutically effective amount of a dopamine precursor and/or a dopa decarboxylase inhibitor.

4. A pharmaceutical composition as claimed in claim 3 wherein the dopamine precursor is levodopa and the dopa  
 10 decarboxylase inhibitor is carbidopa or benserazide.

5. A pharmaceutical composition as claimed in claim 3 or claim 4 wherein the compound of formula I is N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate.

15 6. The use of a pharmaceutical composition containing a pharmaceutically effective amount of a compound of formula I

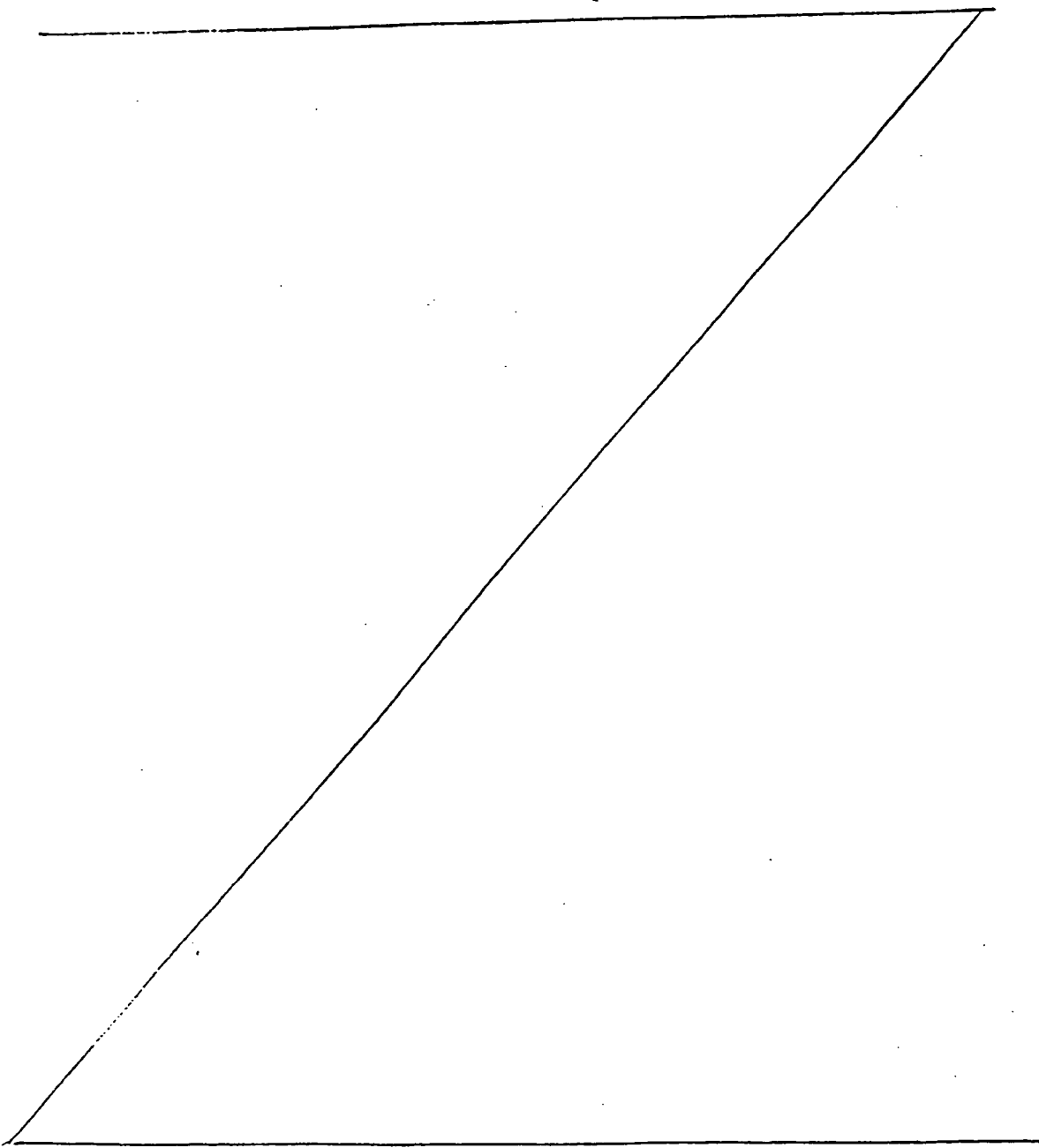


in which Ar is optionally substituted phenyl,  $R_1$  is an optionally substituted aliphatic group and  $R_2$  and  $R_3$   
 20 are H or optionally substituted alkyl groups or  $R_2$  and  $R_3$  together with the nitrogen atom to which they are attached complete a heterocyclic ring and a pharmaceutically effective amount of a dopamine precursor and/or a dopa decarboxylase inhibitor for  
 25 the treatment of Parkinson's disease.

- 21 -

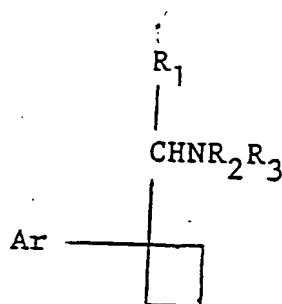
7. The use as claimed in claim 6 wherein the dopamine precursor is levodopa and the dopa decarboxylase inhibitor is carbidopa or benserazide.

5 8. The use as claimed in claim 6 or claim 7 wherein the compound of formula I is N,N-dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate.



- 22 -

9. A pharmaceutical composition for the treatment of Parkinson's disease which comprises a pharmaceutically acceptable diluent or carrier and a pharmaceutically effective amount of a compound of formula I



- in which Ar is optionally substituted phenyl,  $R_1$  is an optionally substituted aliphatic group and  $R_2$  and  $R_3$  are H or optionally substituted alkyl groups or  $R_2$  and  $R_3$  together with the nitrogen atom to which they are attached complete a heterocyclic ring.

10. A pharmaceutical composition as claimed in claim 9 which additionally contains a pharmaceutically effective amount of a dopamine precursor and/or a dopa decarboxylase inhibitor.
11. A pharmaceutical composition as claimed in claim 10 in which the dopamine precursor is levodopa and the dopa decarboxylase inhibitor is carbidopa or benserazide.
12. A pharmaceutical composition as claimed in any one of claims 9 to 11 in which the compound of formula I is N,N, -dimethyl-1-[1-(4-chlorophenyl)cyclobutyl]-3-methylbutylamine hydrochloride monohydrate.



# INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 88/00129

**I. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all) <sup>4</sup>  
 According to International Patent Classification (IPC) or to both National Classification and IPC  
 IPC <sup>4</sup>: A 61 K 31/135; A 61 K 31/395; A 61 K 31/215;  
 A 61 K 31/33; A 61 K 31/195

**II. FIELDS SEARCHED**  
 Minimum Documentation Searched <sup>7</sup>  

Classification System	Classification Symbols
IPC <sup>4</sup>	A 61 K 31/00

 Documentation Searched other than Minimum Documentation  
 to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>

III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup>		
Category <sup>9</sup>	Citation of Document, <sup>11</sup> with Indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
Y	EP, A, 0230742 (THE BOOTS CO.) 5 August 1987 see claims 1-3,10 cited in the application --	1-5,9-12
Y	"Lehrbuch der Pharmakologie und Toxikologie", 1982, editor H. Bader, Edition Medizin, (Weinheim, DE), pages 125-127 and 208 see page 208, paragraph B.4.3 --	1-5,9-12
Y	DE, A, 3212682 (THE BOOTS CO.) 21 October 1982 see page 26, last three lines; page 27, lines 1-4; pages 45-46 & GB, A, 2098602 (cited in the application) --	1-5,9-12
Y	EP, A, 0191542 (THE BOOTS CO.) 20 August 1986 see page 30, lines 5-13; pages 55-56 cited in the application --	1-5,9-12
	./.	

- \* Special categories of cited documents: <sup>10</sup>
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

## IV. CERTIFICATION

Date of the Actual Completion of the International Search

11th May 1988

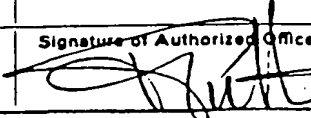
International Searching Authority

EUROPEAN PATENT OFFICE

Date of Mailing of this International Search Report

15 JUN 1988

Signature of Authorized Officer

 P.C.G. VAN DER PUTTEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	EP, A, 0108488 (THE BOOTS CO.) 16 May 1984 see claims 9,10,22-24; pages 19-20 & GB, A, 2127819 (cited in the application) --	1-5,9-12
Y	EP, A, 0111994 (THE BOOTS CO.) 27 June 1984 see pages 22-23 & GB, A, 2128991 (cited in the application) --	1-5,9-12
Y	The Merck Index, an Encyclopedia of Chemicals, Drugs, and Biologicals, 10th Edition, 1983, Merck & Co., Inc. (Rahway, N.J., US), page 1054 see monograph 1048  -----	1-5,9-12

## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>1</sup>

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 6-8 because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv)

Methods for treatment of the human or animal body by means of surgery or therapy, as well as diagnostic methods.

2. ☒ Claim numbers XX because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

XX Claims 3,5,10,12 searched incompletely

A compound cannot be sufficiently characterised by its metabolism or its biological or biochemical activity.

3. ☐ Claim numbers..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>2</sup>

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 8800129  
SA 20936

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 06/06/88. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0230742	05-08-87	AU-A- 6644286	18-06-87
		GB-A- 2184122	17-06-87
		JP-A- 62155240	10-07-87
DE-A- 3212682	21-10-82	BE-A- 892758	05-10-82
		FR-A, B 2504920	05-11-82
		NL-A- 8201347	01-11-82
		JP-A- 57181043	08-11-82
		AU-A- 8221382	14-10-82
		LU-A- 84070	07-06-83
		GB-A, B 2098602	24-11-82
		US-A- 4522828	11-06-85
		AU-B- 545595	18-07-85
		CH-B- 652117	31-10-85
		SE-B- 452611	07-12-87
		US-A- 4746680	24-05-88
		US-A- 4443449	17-04-84
EP-A- 0191542	20-08-86	AU-A- 5172585	24-07-86
		JP-A- 61197548	01-09-86
EP-A- 0108488	16-05-84	AU-A- 1922383	05-04-84
		GB-A, B 2127819	18-04-84
		AU-A- 1922483	12-04-84
		JP-A- 59089659	23-05-84
		EP-A, B 0111994	27-06-84
		GB-A, B 2128991	10-05-84
		US-A- 4629727	16-12-86
		AU-B- 557248	11-12-86
EP-A- 0111994	27-06-84	AU-B- 561772	14-05-87
		GB-A, B 2127819	18-04-84
		AU-A- 1922483	12-04-84
		JP-A- 59084847	16-05-84
		US-A- 4629727	16-12-86
		AU-B- 557248	11-12-86
		AU-A- 1922383	05-04-84
		EP-A, B 0108488	16-05-84
		JP-A- 59089659	23-05-84

GB 8800129  
SA 20936

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 06/06/88. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0111994		GB-A, B 2128991 AU-B- - 561772	10-05-84 14-05-87
-----			

1/9/1  
DIALOG(R) File 351:Derwent  
(c) 2000 Derwent Info Ltd. All rts. reserv.

003542633

WPI Acc No: 1982-90626E/198243

Related WPI Acc No: 1982-88067E

**Antidepressant 1-phenyl-1-aminomethyl-or aminoethyl-cyclobutane derivs -  
prepd. e.g. by reductive amination of 1-phenyl-cyclobutyl or  
-cyclobutylmethyl ketone(s)**

Patent Assignee: BOOTS CO LTD (BOOT ); BOOTS CO PLC (BOOT )

Inventor: JEFFERY J E; KOZLIK A; WILMSHURST E C

Number of Countries: 022 Number of Patents: 034

Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
DE 3212682	A	19821021				198243	B
GB 2098602	A	19821124	GB 829591	A	19820401	198247	
NL 8201347	A	19821101				198247	
NO 8201087	A	19821101				198247	
FR 2504920	A	19821105				198250	
JP 57181043	A	19821108	JP 8257178	A	19820406	198250	
FI 8201197	A	19821130				198301	
PT 74580	A	19821230				198308	
SE 8202166	A	19830228				198311	
DK 8201464	A	19830228				198315	
ES 8305678	A	19830716				198339	
HU 26666	T	19830928				198345	
US 4443449	A	19840417	US 82365287	A	19820405	198418	
ES 8403097	A	19840601				198429	
GB 2098602	B	19840822				198434	
DD 208348	.	19840502				198435	
ES 8406413	.	19841101				198503	
ES 8406414	A	19841101				198503	
ES 8407002	A	19841116				198505	
RO 84802	A	19841030				198518	
CH 652117	A	19851031				198547	
CS 8202457	A	19850831				198548	
CS 8301735	A	19850831				198548	
AT 8201325	A	19860815				198638	
IL 65257	A	19860530				198639	
RO 89436	A	19860630				198703	
SE 452611	B	19871207				198751	
CA 1248955	A	19890117				198910	
SU 1461372	A	19890223	SU 3834158	A	19850109	198935	
JP 89041132	B	19890904				198939	
SU 1482522	A	19890523	SU 3426748	A	19820405	198948	
DE 3212682	C	19920326	DE 3212682	A	19820405	199213	
IT 1235758	B	19920928	IT 8248157	A	19820402	199313	
NL 192201	B	19961101	NL 821347	A	19820331	199648	

Priority Applications (No Type Date): GB 8110710 A 19810406; GB 8110709 A 19810406

Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
DE 3212682	A		103		
DE 3212682	C		32		
NL 192201	B		28	C07C-211/26	
IT 1235758	B			A61K-000/00	

Abstract (Basic): DE 3212682 A

1-Phenyl-1-(aminomethyl or aminoethyl) cyclobutane derivs. (I) and their salts are new, (where n is 0 or 1; R1 is when n is 1 is H or 1-3C alkyl, or when n is 0 is 1-6C alkyl, 3-7C cycloalkyl, (3-6C cycloalkyl)-(1-3C alkyl), 2-6C alkenyl, 2-6C alkynyl, or R9, R10-phenyl in which R9 and R10 are H, halogen or 1-3C alkoxy; R2 is H or 1-3C alkyl; R3 and R4 are H, 1-4C alkyl, 3-6C alkenyl, 3-6C alkynyl, 3-7C cycloalkyl or a gp. -CO-R11 in which R11 is H (sic), or N(R3)(R4) is an opt. substd. 5- or 6-membered heterocyclic ring opt. contg. further heteroatoms; R5 and R6 are H, halogen, CF3, 1-3C alkyl, alkoxy or

alkylthio, or phenyl, or R5 and R6 together with the C-atoms to which they are attached form a second benzene ring which is opt. substd. by one or more halogen atoms or 1-4C alkyl or alkoxy groups or to which is fused a further benzene ring; and R7 and R8 are H or 1-3C alkyl). Intermediates of formulae (V) (provided that R5 is other than H when R1 is CH3 or C2H5), (VI) and (XVII) are new and claimed.

As indicated by reversal of reserpine-induced hypothermia in mice, the new cpds. are antidepressants.

Abstract (Equivalent): GB 2098602 B

Compounds of formula (I) in which n = 0 or 1; in which, when n = 0, R1 is a straight or branched chain alkyl group containing 1 to 6 carbon atoms, a cycloalkyl group containing 3 to 7 carbon atoms, a cycloalkylalkyl group in which the cycloalkyl group contains 3 to 6 carbon atoms and the alkyl group contains 1 to 3 carbon atoms an alkenyl group or an alkynyl group containing 2 to 6 carbon atoms or a group of formula (II) in which R9 and R10 which are the same or different, are H, halo or an alkoxy group containing 1 to 3 carbon atoms; in which, when n = 1, R1 is H or an alkyl group containing 1 to 3 carbon atoms; in which R2 is H or an alkyl group containing 1 to 3 carbon atoms; in which R3 and R4, which are the same or different, are H, a straight or branched chain alkyl group contg. 1 to 4 carbon atoms, an alkenyl group having 3 to 6 carbon atoms, an alkynyl group having 3 to 6 carbon atoms, a cycloalkyl group in which the ring contains 3 to 7 carbon atoms, a group of formula R11CO where R11 is H or R3 and R4 together with the nitrogen atom to which they are attached form an optionally substituted heterocyclic ring having 5 or 6 atoms in the ring which opt. contains further hetero atoms in addition to the nitrogen atom, in which R5 and R6, which are the same or different, are H, halo, trifluoromethyl, an alkyl group contg. 1 to 3 carbon atoms, an alkoxy or alkylthio group containing 1 to 3 carbon atoms, phenyl or R5 and R6, together with the carbon atoms to which they are attached, form a second benzene ring optionally substd. by one or more halo, alkyl or alkoxy groups contg. 1 to 4 carbon atoms or the substituents of the second benzene ring together with the two carbon atoms to which they are attached form a further benzene ring; and in which R7 and R8 which are the same or different are H or an alkyl group contg. 1 to 3 carbon atoms; and their pharmaceutically acceptable salts.

Abstract (Equivalent): US 4443449 A

Arylcyclobutylalkylamines of formula (I) and its salts are novel. In (I), R1 is H or 1-3C alkyl; R2 is H or 1-3C alkyl; R3 and R4 are H, opt. branched 1-4C alkyl, 3-7C cycloalkyl or R3 and R4 together with N-atom attachment form heterocyclic ring selected from pyrrolidinyl, piperidinyl and piperazinyl, each opt. substd. by 1 or more methyl gps., and 1,2,3,6-tetrahydropyridyl; R5 and R6 are H, halo, 1-3C alkyl or -CF3 with the proviso that one of R5 and R6 is halo, or R5 and R6 together form a benzene ring with C-atom to which they are attached. R7 and R8 are H or 1-3C alkyl. Specifically claimed are cpds. of formula (II) and (III).

(I) are used to treat depression and are administered in daily doses of 1-1000 (5-500) mg of (I). (14pp)a

Title Terms: ANTIDEPRESSANT; PHENYL; AMINOMETHYL; AMINOETHYL; CYCLOBUTANE; DERIVATIVE; PREPARATION; REDUCE; AMINATE; PHENYL; CYCLOBUTYL; CYCLOBUTYL; METHYL; KETONE

Derwent Class: B05

International Patent Class (Main): A61K-000/00; C07C-211/26

International Patent Class (Additional): A61K-031/13; A61K-031/135; A61K-031/395; C07C-013/06; C07C-033/34; C07C-047/11; C07C-047/23; C07C-049/21; C07C-049/22; C07C-069/61; C07C-087/45; C07C-091/28; C07C-093/14; C07C-095/02; C07C-103/30; C07C-103/36; C07C-103/37; C07C-103/38; C07C-121/00; C07C-149/42; C07C-211/27; C07C-217/10; C07D-057/26; C07D-207/06; C07D-211/70; C07D-295/00; C07D-295/02; C07D-295/04; C07D-295/06; C07D-521/00

File Segment: CPI

Manual Codes (CPI/A-N): B07-H04; B08-D02; B10-A15; B10-A24; B10-B03B; B10-B04B; B10-D01; B10-D03; B10-F02; B12-C06

Chemical Fragment Codes (M2):

\*01\* G002 G010 G011 G012 G013 G014 G015 G016 G019 G020 G021 G030 G038  
G111 G112 G113 G221 G331 G341 G543 H541 H542 H594 H599 H601 H602  
H603 H604 H608 H641 H642 H685 H689 K0 L1 L144 M1 M111 M113 M119 M210

M211 M212 M213 M214 M231 M232 M233 M240 M271 M272 M280 M281 M282  
M283 M311 M320 M321 M322 M344 M353 M391 M392 M414 M510 M520 M531  
M532 M533 M541 M710 M903

\*02\* G002 G010 G011 G012 G013 G014 G015 G016 G019 G020 G021 G030 G038  
G039 G050 G111 G112 G113 G221 G331 G341 G530 G543 G553 G563 G573  
G599 H541 H542 H543 H594 H599 H601 H602 H603 H604 H608 H609 H641  
H642 H643 H685 H689 H714 H721 H731 J451 J581 M1 M111 M113 M119 M123  
M126 M131 M135 M210 M211 M212 M213 M214 M215 M216 M231 M232 M233  
M240 M262 M271 M272 M280 M281 M282 M283 M311 M312 M313 M320 M321  
M322 M323 M331 M332 M340 M342 M344 M353 M372 M391 M392 M414 M510  
M520 M531 M532 M533 M541 M542 M710 M903

\*03\* G002 G010 G011 G012 G013 G014 G015 G016 G019 G020 G021 G030 G038  
G039 G111 G112 G113 G221 G331 G341 G530 G543 G553 G563 G573 G599  
H541 H542 H543 H594 H599 H601 H602 H603 H604 H608 H609 H641 H642  
H643 H685 H689 H721 H731 J471 J581 M1 M111 M113 M119 M123 M126 M132  
M135 M210 M211 M212 M213 M214 M231 M232 M233 M240 M262 M271 M272  
M280 M281 M282 M283 M311 M312 M313 M314 M315 M316 M320 M321 M322  
M323 M331 M332 M333 M340 M342 M343 M344 M353 M372 M391 M392 M414  
M510 M520 M531 M532 M533 M541 M542 M710 M903

\*04\* G002 G010 G011 G012 G013 G014 G015 G016 G019 G020 G021 G030 G038  
G039 G111 G112 G113 G221 G331 G341 G530 G543 G553 G563 G573 G599  
H100 H102 H103 H161 H181 H541 H542 H543 H594 H599 H601 H602 H603  
H604 H608 H609 H641 H642 H643 H685 H689 H716 H721 H722 H731 H732  
J011 J371 M1 M111 M113 M119 M123 M126 M129 M132 M135 M143 M210 M211  
M212 M213 M214 M215 M216 M231 M232 M233 M240 M271 M272 M273 M280  
M281 M282 M283 M311 M312 M313 M314 M315 M316 M320 M321 M322 M331  
M332 M333 M340 M342 M343 M344 M353 M373 M391 M392 M414 M510 M520  
M531 M532 M533 M541 M542 M543 M640 M650 M710 M903 P451

\*05\* F010 F011 F012 F013 F014 F015 F020 F021 F423 F432 F433 F553 G002  
G010 G011 G012 G013 G014 G015 G016 G019 G020 G021 G030 G038 G039  
G111 G112 G113 G221 G331 G341 G530 G543 G553 G563 G573 G599 H1 H181  
H182 H2 H201 H202 H541 H542 H543 H594 H599 H601 H602 H603 H604 H608  
H609 H641 H642 H643 H685 H689 H721 H731 M1 M111 M113 M119 M123 M126  
M132 M135 M210 M211 M212 M213 M214 M231 M232 M233 M240 M271 M272  
M280 M281 M282 M283 M311 M312 M313 M314 M315 M316 M320 M321 M322  
M331 M332 M333 M340 M342 M343 M344 M353 M373 M391 M392 M413 M510  
M521 M531 M532 M533 M541 M640 M650 M710 M903 P451

Derwent Registry Numbers: 0246-S; 0330-S; 0341-S; 0347-S; 0358-S; 0918-S;  
1044-S; 1053-S

?